

Figure 1: Isolation of monokaryotic strain deficient in laccase activity.

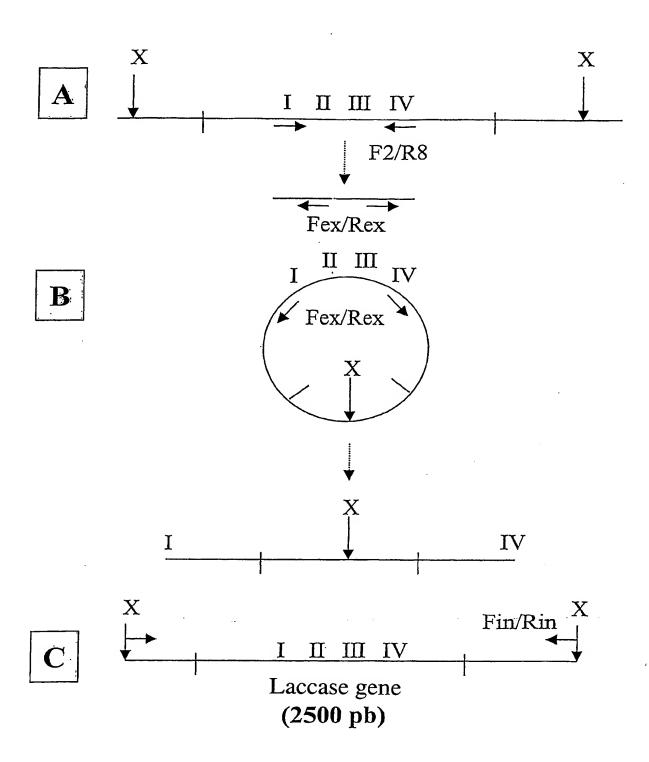


Figure 2: Isolation of the gene coding for the laccase of *Pycnoporus cinnabarinus* laccase.

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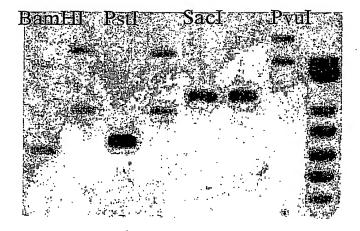


Figure 3: Southern blot study of the gene coding for the laccase of *Pycnoporus cinnabarinus*.

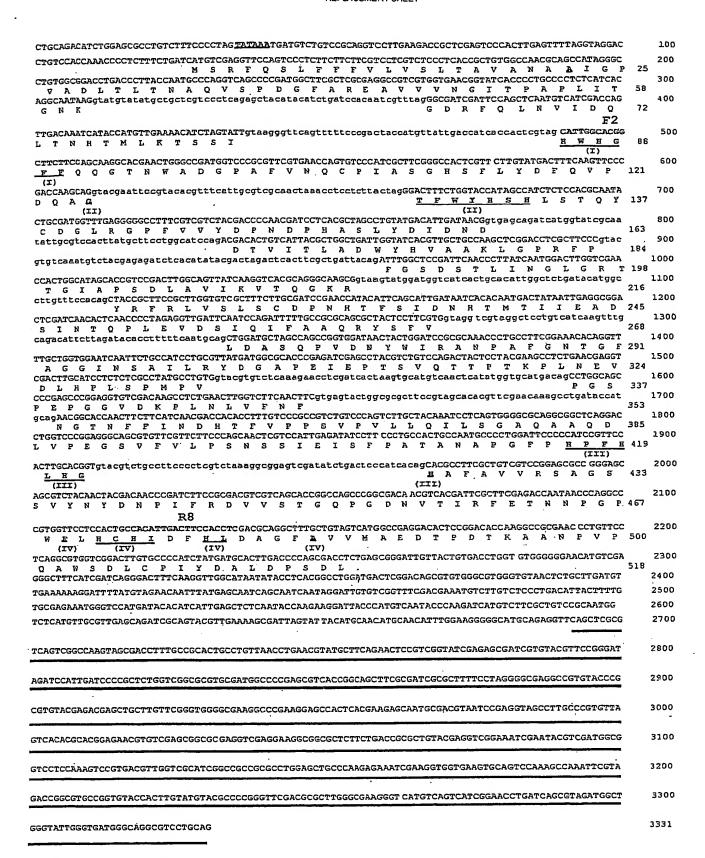


Figure 4: Sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus*

AGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCAGGGCAGCGACGTA CCGGCCGGCGTGCGCCATTGAGGTACATGAGCCGGGGGGGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCG CGGCGATGAAACGTTTCCCACCATTGGGAAGAAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGC GGCGTCGCGCCTTTGCCGCAGAGGCCGGCGGCGACATGCACAGCGAAGGTCCGTTGCGGATGGGAAGCAGG CAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGAGCGTAGGCGCCCTCCCATAAGGCGGCAAGCATC ATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACC ATCGGCATCCTCAGCCTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGC TTCTTGGATGATCATGTCGTAACTTTTTCTGACCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGT ATGCCTCCCATTCATAAACGATAACCCCTTCCTTCAGGTTGGTCATCTCCATAGAGCGGCACGCTCTCAAGG CCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCATGGGATC ACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGT TCAGTCACCACATGGCAAAAAAGCTGCACCATACTCTTTATGGTGAGTTGTTCGTGAGTGGTATACAGTCAT TCATGAGGGAATGCCCACCGGATAGGGTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGT CCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAAACAGCGTTGACTGTGAATCCCTGGTGTGT GTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTGCGAGCGGCACAG GCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGG AGCGAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGAT TGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAAGTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGT CTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAATGCGCACACACGGAGGCTTTA CATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCT CTCATGACGTCCGCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATGGTAGTGGA GTCAGCCTGGCCAGTGCGTAGTCCCGTCTCTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTGC TTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTGCACGCCCAGAGGTATTGGGCTCGTCA GATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCTATAGCCCTT CTTCGCGCGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTAC CGAACAATTGACTTACCGACATCCTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCC GCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGACGGCCGCTCATCAGGACCAGACCAGTCTCAAT GTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTGGTGCACCGTCGCTTAC GATCATG

Figure 5 : Sequence of the promoter sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus* (up to the ATG coding for the methionine of the laccase).

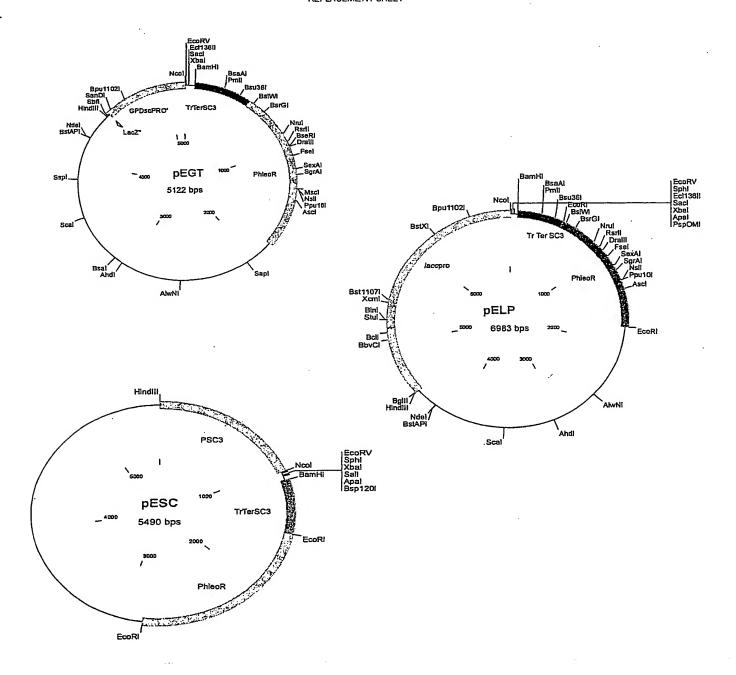


Figure 6: Restriction map of the three expression vectors used for the production of laccase in *Pycnoporus cinnabarinus*.

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCCCTTAAGACGCGTGGATCCGCAGGTGAAC GCGCCTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGT CATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAGGTGTTGACTCATTCCCTCGATAATGAACATTGTTGTTGTTTG TTCTCTATCCGCTCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAG GGGGGTACAAAAGGAGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCT CAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATC GTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACG AGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTTCACGAAGCTCTTGCTGATCGCGATGCCG GGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGTGTTGGTCGGCGTCGG CTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCAC CCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACACCGGC CCCCTCGAGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGTCCTTCTCGCACC TGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAGGCTCTCGCGC CACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGGGAATTCGT AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACATACGAGCCGGAAGCATAAAGTGTAAAG CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCT GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACG AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC ACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCC TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAA AACTCACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTAAATTAAAATGAAGTTTTAAATCAA TCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTC ATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG TGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATT GGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGA TCTTCAGCATCTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAAATGCCGCAAAAAAGGGAATAAGGGCGAC ACGGAAATGTTGAATACTCATACTCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCA TGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGC AGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGG TGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTA AGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTA CGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC GCGGGCCCCGCCGCCCCCTCGGGCGAGCGGGTGTATCTACGAACGGAACTGGGAGGCGACTCGGAAGAGTTTGGTTAGAAAGGG GAACACCATCGCGGACGGCCCAGTGCTCTGGDCAGCTGAGCGTGCATTGTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC GGGATCGGAGGGTGGCGCGAGAGCCTCTTCGGTGTGAGATTAGTAACTGTACTGCGAAGCCGCGGAGGGGTTAGGATGAGAGGTAG ACAGGGTCGCAGCCCAGGTGCGAGAAGGACTGCGAAGGACTGTTCTTCGACCGCGCACCTGCAATTGCGCGCATGGATAGAATAGA GCCGCCGCTGTTTCATCCCGTTTTGTCAGTATCGATCGGATCTTTCGGGCGTGGGTATAAAAGCGCGCCGCCGCCGTCTCCCT CTTTCTCCAGCACTCCCATCCAGAGCACTTCCCTCTCCCATCGCATCACCACACAATAATGCCCATCAC

Figure 7: Nucleotide sequence of the vector pEGT, containing the gpd gene promoter (4480-5112), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507).

AGCTTCTCCGGCCCCGAATCGAACGGCAGGATGTGTGGGCGTGTCCAATATTGCCATGAAAATCTGTCAGAAGTGAGCCCTCTCGTCAC CCTGTACAGCTTCGCTGAGTTGAAAAGCAGGGTTCATCTTGGGCTCACTGATGCACTGAGCTCGACCGGAGAACTAAATGACCAGCCGG AGTGTTCACTAACTTAACGCCGGGTATTCAGGGCAGCTTCTCTATGTTGCGCCTACGACGTAGATCACCGCCCATGAACGGGGGAAACG GGGAGGGGTGCGTTTGGTACGTCTTACGTCTGGCTATGTTGTATTGACCAGCGTCTGCAGAAGATGGGCACGACGATGCGCCGAGCCG AGGGGCTTAGATGGAGAGTGACACGTCTGAGCTCCCCAACACGCCTTCGCCGAGGGTGCGTCTCCGCGGACATTCACCTCAGTTCATTG TTCTGACCTGCCTAATTGTATAGACCGGCCAACAACCTTGCTGACGCCCATCATAACAGTGCCCTGCACAGAGCCTTCCCACTCAGTCGG CGCCTCCCTCAATCAATCCCACTAACTCGCCGGCTCTGCCCCTTCGCCGCTCGACACGTCGCTTGGAAGAGCCCGGGCACGGGCGTCCGC AACGCGCGGAAGAAAATAATTTACGGGAGCCTCCCCAGGTATAAAAGCCCCTCACCCGCTCACTCTTTCTCCAGTCGAACACCCCAGT TCAACTACCCAGCCCTTCCTTCCTTCGCTATCCTTCYTTACAACCTGCTCGCCATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGAC GGGCCCGGTACCGCGCCCCTTAAGACGCGTGGATCCGCAGGTGAACGCGCCTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGC AATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAG TTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGTGGACTAAAGCACTCCACTTTACGGCGAGGATGC CAGTCTACGTCATGAATGAAGCCTCAGGTCCCGAAGTAAGGGGGGTACAAAAGGAGGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCA CCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCG CATACATGCACGACTTCAAAACATCCATTCTATCATTTTGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCCTTTCTTCTT TTACTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAG AGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTT CACGAAGCTCTTGCTGATCGCGATGCCGGGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGCCGCGTC ACAGCTCGTCCAGGCCGCGCACCCCAGGCCAGGCCAGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACG GCCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGT CCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAG GCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGG GAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTG TAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA GCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTCACTGACTCGCTGCGCTC GGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAAC ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGC ATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTG CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTA ACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTT TTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTT GGGAAGCTAGAGTAAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTG GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTC CTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGT AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAAT ACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTAC CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAA AAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTTTCAATATTATTGA AGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCC CGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGC GCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGAC CATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGA AGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCA

Figure 8: Nucleotide sequence of the vector pESC, containing the sc3 gene promoter (1-1033), a phleomycin resistance marker (1540-2855) and the sc3 gene terminater (1104-1540).

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCCTTAAGACGCGTGGATCCGCAGGTGAACGCGC CTATCGGTGGGATATTCGGGCGACGGGAGCCTCGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGT TCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGT GTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACG $\verb|CCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATCATTTTGGGATCGTACAATTATTAGA$ CATGTTGTACAACGTTACATTCCTTTCTTCTTTTACTCCCGGCCCAGTCTATGTAGAGGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTT AGAGCGCCGCCTTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGCGGGGAGTAGCCCC ACCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCGGTCAGTCCTGCTCCTCGGCCACGAAGTGCACGCAGTTG GACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGG TGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATGGGAGTGCTGGAGAAAGAGGGGAGACGGCGGGGGGGCGCCCTTTTATACCCACG CCGTCGGGCGCCACCACCAGCCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGA AGAACAGTCCTTCGCAGTCCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTA ATCTCACACCGAAGAGGCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTC ARATCAGATCCCCGGGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAAATTCCACAACATACGAGCC AACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTG CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG TTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTTGG TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT ${\tt CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAA}$ GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACA GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTTTTCAATATTATTGAAGCATT TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAG TGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTTTCGG TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA GGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG TGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGT GCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGGCCAGTGCCAAGCTTAGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCA CGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCGCGGCGATGAAACGTTTCCCACCA TTGGGAAGAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGCGGCGTCGCGCCTTTGCCGCAGAGGCCGGCGGCGACATGCA

Figure 9: Nucleotide sequence of the vector pELP, containing the laccase gene (promoter 4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507) (continuation of the sequence on the following page).

CAGCGAAGGTCCGTTGCGGATGGGAAGCAGGCAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGAGCGTAGGCGCCCTCCCA TAAGGCGGCAAGCATCATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACCATCGGCATCCTCAGC CTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGCTTCTTGGATGATCATGTCGTAACTTTTTCTGA CATAGAGCGGCACGCTCTCAAGGCCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCAT GGGATCACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGTTCAGTCACCA GTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGTCCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAA ACAGCGTTGACTGTGAATCCCTGGTGTGTGTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTG CGAGCGGCACAGGCAGCCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGGAGC GAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGATGTGAAAGGCTCCATCATAT GTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGTCTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAA CCCAGCATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCTCTCATGACGTCC GCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATCGTAGTTGGAGTCAGCCTGGCCAGTGCGTAGTCCCGTCT CTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTGCTTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTG CACGCCCAGAGGTATTGGGCTCGTCAGATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCT CGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTACCGAACAATTGACTTACCGACATC CTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCCGCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGA CGGCCGCTCATCAGGACCAGACCAGTCTCAATGTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTG

Figure 9: Nucleotide sequence of the vector pELP (continuation), containing the laccase gene (promoter4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene ternminater (71-507).

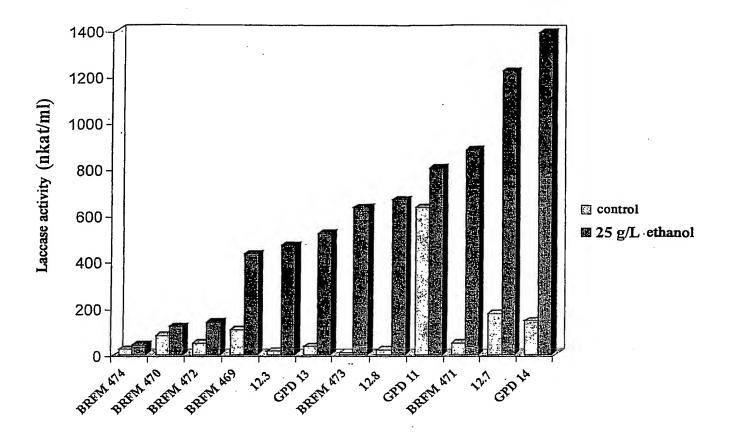
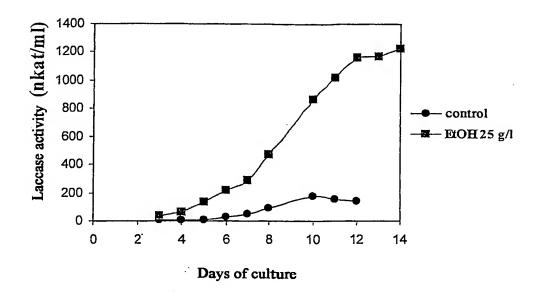


Figure 10: Results of production of the transformants having the most significant activities. The culture was carried out with or without (control) ethanol.



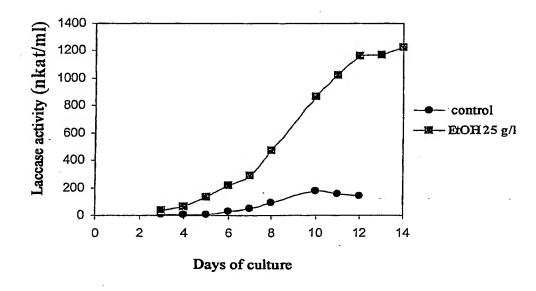


Figure 11: Monitoring of the laccase activities of the transformants GPD 14 and 12.7 as a function of time with or (control) without ethanol

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tggggartggttctatatatatcaaaatgatcttctgtcctgagctttcctcgtccttgttttcgtcttgtcgtgctgccgcgacatgctttattaaaccat
\tt TGGCGAGCTGCCCGCGCCCAAGGAGATAGCATAATCGCCTGAGAAACCTAGTCGTCTCATGGCCGTGTAACCGTTCTTGCGACTTATTTTCGCACTTCTC
{\tt TCAGARTATAAAGGCCTATTGTGATACGGTTCATCTAACCCCAGCGTCCCCTCCGAAAGATGGGCTGCCTCTCACTCTTCGCATTCCTTACTGCTTTAAA
                                                                            300
CTCAGTTCATGCCGCTGTGGGTCCCGTTACGACTTAACACTGATCGTAGATACTGTCGCCCCGACGGTGCTGCTTTCGCCCGGGAAGGTGAGACTTTG
S V B A A V G P V T D L T L I V D T V A P D G A A F A R E
CGACTGTAAATGCCGGATTTGAGTTTCTAATTATAATCTTCCAGCCATTGTCGTCCAAGAGGAACCAAACTCCGTCATTGGTCCGGTCATCGTAGGTGGG
                                                                            500
                                     V V O E E P N S V I G P
                                                                             60
600
L D S P N M R Q S T S I H W H G I F Q G N
GACTCGAATATAGGTCAGAATTGGGCTGGTGGGTTGGCCTTCCTGAAGCCTGCTCGAATTTATCTTCCTGAATTTTTAGATGGCGCCGCATTCGTTAACC
                                                                            800
                                                                            107
ag<u>gtaagg</u>agatgitectgecttegetttececagaactaattatec<u>ta</u>gtgee ecattgeeeeegggggggetegttettgtaegactttaeegaacet
                                     CPIAPGGDSFLYDFTEP
FQTGTFWYHSHLSTQYCDGLRGAFV
                                                                            150
CCGCTTTCTTCTCACTTATCTAGATCCACCTCGCTCGACCCTTACCGGTTGCTCTACGATGTCGACGAGTCGACTGATTACTCTGGCGGACTG 1100
                  I Y D P L D P Y R L L Y D V D D E S T V I T L A D W
GTACCACAGCTATGCGGAGGACATTCTAATCGC<u>GTAGGA</u>GATTTTCCCAAGATGTCTCCTCTGCCTCTCTGAAATCCATGAAC<u>TAG</u>TGCAGGCGACACTA 1200
 YHSYAEDILIA
                                                                  AGDT
                                                                            191
TCCTCATCAATGGTCACGGAAGATTCGCCGGAGCCGGCGGAACGGCAACAGAACTATCTGTCATTACTGTTGAGCATGGAAAGCGGTAGGCATTCTCCCT 1300
  LINGHGRFAGAGGTATELSVITVEHGKR
CGGCTTTGTAGATGTGTCTAATTTGTGATAGCTACCGATTGCGATTTGCCAATATCGCTTGTGACCCTTGGTTTGCCGTGAAAATCGATAGCCATACGAA 1400
                        YRLRFANIACDPWFAVKID
                                                                            243
CCTTCGCGTTATCGAAGCTGACGGTATTACTACTGTGCCTGTCACGGTGGACTCCTTCAATGTAGGCTTACCCTTAGCACTTTCCCACTCTGGATCCTCT 1500
   RVIEADGITT
TAT GACTTCCCAAGATCTTTGTGGGCCAACGAT ATAGTGTCATCCTCCATGCCAACCAGCCTGTTGGAAACTACTGTAAGCTGCCTAAATGTTGCATGAC 1600 I F V G Q R Y S V I L H A N Q P V G N Y
TGTCCATGATTCTAACCCCGCCAGGGATTCGGGCCGCTCCGAACGGCGTGAGCAATTTCGCGGGTGGGATCGACTCGGCTATTCTCCGTTATGTTGGCGC
                  WIRAAPNGVSNFAGGIDSAILRYVGA300
CCCAGAAGAAGACCCCAACACTAGTGAGGATACTCCATCCGACACACTTCAAGAGCAGGATCTTCACCCGCTGATCCTACCCGGCGCCCAGGCATCCAC 1800
 PEEEPNTSEDTPSDTLQEQDLHPLILPGAPGIH
                                                                            333
TCCCGTGGGGCCGCCGACGTTGTCCACACCGTATCAAT GGAGTTTGTGAGTGT GGCGACTTTTCTGGCCCCCCTTTATT AATAT AATCTGGTTAGGATGGC 1900
 SRGAADVVHTVSMEF
                                                                            348
GCAAACTT CCAATTCCTCCTGGATGGCGTGGCCTTCCAGCCGTGCGTCATCTCTTTCAAAGAATTTATCTAGCTGACGATTTTGAAATGTAGCCCGACCA 2000
TGCCCGTCCTTCTGCAAATATTATCGGGAGCGCAGACTGCTAATACCCTTCTCCCGGCGGGATCCTTTATCCAAGCGTCGCACAATGACATCGTGGAGCT 2100
       LQILSGAQTANTLLPAGSFIQASHNDIV
GGTTCAATCCC<u>GTATTT</u>TCATTCGACTTCCATAAGATGACGATGGCTCACTATGGTTTTTACC<u>CAG</u>CCTCGCAGAGATGTCGTATCCACCGGTACCGATC 2400
                                                                            441
TIRFRADNP
CTGAATCTCTCGTTGTCTTTGGTTCTCATAATCTCATCAGAGGTCCATGGTTCCTTCACTGCCACATTGACTCGGCCTTGAACTCGGCTTTGCTTTGGT 2600
                                   WFLHCHIDWHLELGFALV
GATTGCAGAAGCGCCTAGCGAATGGGACAGCGACATTAACCCTCCTGGTGCGCTGCCTGTGAACCTTTCTCCCTACACTTGCTAAGATCGCTCTAGCTG 2700
IAEAPSEWDSDINPP
CGT GGGAT GACCT ATGCC CTACGTTCGCTTTGGCTTCTCTTTTACTATTTCAAGTTTCCTCACATTCTC AACTTCACAGATATGATGCCCTGCCGCCTGAG 2800
CAGCAGTAATCGAGTTAAGAACCTCAACGTTGACTAAGGAAAAAGCAAAGCAGAATATGAAACTCTCATTTATTCTTATATCGACACATTCACTATTCAA 2900
ART ATATA CATAA CGTCCGTGGGGTTAGTTAATTCGT
                                                                           3037
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Gene of the laccase of Halocyphina villosa